PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

DOUGLAS P. CERRETTI

Appln. No.: **08/538,709** Group Art Unit: **1647**

Filed: **October 3, 1995** Examiner: **Draper, G.**

For: **DNA ENCODING CYTOKINE DESIGNATED LERK-6**

SUPPLEMENTAL INFORMATION DISCLOSURE
STATEMENT UNDER 37 C.F.R. §§ 1.97 and 1.98

Assistant Commissioner
for Patents
Washington, D.C. 20231

Sir:

Supplemental to the Information Disclosure Statement filed June 30, 2000, and in accordance with the duty of disclosure under 37 C.F.R. § 1.56, Applicant hereby notifies the U.S. Patent and Trademark Office of the document which is listed on the attached Form PTO-1449 which the Examiner may deem relevant to patentability of the claims of the above-identified application.

A copy of the listed document is provided herewith.

For the Examiner's convenience, also attached hereto, please find the Suspension Notice dated September 3, 1998, Notice of Allowability dated January 20, 1998 and an Amendment and Response dated November 26, 1997 for cited U.S. Appln. No. 08/393,462. These documents are submitted in support of the allowed claims for said application.

The present Supplemental Information Disclosure Statement is being filed after three months from the application's filing

date, and after the mailing date of the first Office Action on the merits, therefore the required fee under 37 C.F.R. § 1.17(p), in the amount of $180.00, is submit herewith.

The submission of the listed document is not intended as an admission that any such document constitutes prior art against the claims of the present application. Applicant does not waive any right to take any action that would be appropriate to antedate or otherwise remove any listed document as a competent reference against the claims of the present application.

Respectfully submitted,

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Paper No.

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Status Inq

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PATENT PUBLICATION

In re Application of
John G. Flanagan, et al.
Application No. 08/393,462
Filed: Feb. 27, 1995
Attorney's Docket No. HMV-011.02.

NOTICE

The purpose of this communication is to inform you that the above - identified application, which has received a patent number or an issue date, is being withdrawn from issue pursuant to 37 CFR 1.313.

The application is being withdrawn for the following purpose: To institute an interference. This withdrawal was requested by the Group Director. Any questions concerning this withdrawal should be addressed to the Group Director.

The issue fee is refundable upon written request. However, if the application is again found allowable, the issue fee may be applied toward payment of the issue fee in the amount identified on the new Notice of Allowance and Issue Fee Due upon written request. This request and any balance due must be received on or before the due date noted in the new Notice of Allowance in order to prevent abandonment of the application.

This application is being returned to the Office of the Director of Group 1600.

Telephone inquiries concerning this matter may be directed to the Director's Office at (703) 308-0196.

Karna Cooper
Paralegal Specialist
Office of the Director
Office of Patent Publication

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

APPLICATION NO	FILING DATE	FIRST NAMED APPLICANT		ATTORNEY DOCKET NO
08/373,462	02/23/95	FLANAGAN	J	HMV-011.02

EXAMINER
SORENSEN, K.

18N2/0120

PATENT GROUP C/O
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ART UNIT 1812 | PAPER NUMBER 12

DATE MAILED: 01/20/98

NOTICE OF ALLOWABILITY

PART I.

1 ☑ This communication is responsive to 12/1/97

2 ☐ All the claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice Of Allowance And Issue Fee Due or other appropriate communication will be sent in due course

3 ☑ The allowed claims are 54-76 to ISSUE AS 1-23

4 ☐ The drawings filed on _____ are acceptable.

5 ☐ Acknowledgment is made of the claim for priority under 35 U.S.C. 119. The certified copy has [ ] been received. [ ] not been received. [ ] been filed in parent application Serial No. _____, filed on _____.

6. ☑ Note the attached Examiner's Amendment.

7 ☑ Note the attached Examiner Interview Summary Record, PTOL-413.

8 ☑ Note the attached Examiner's Statement of Reasons for Allowance.

9 ☐ Note the attached NOTICE OF REFERENCES CITED, PTO-892.

10 ☐ Note the attached INFORMATION DISCLOSURE CITATION, PTO-1449.

PART II.

A SHORTENED STATUTORY PERIOD FOR RESPONSE to comply with the requirements noted below is set to EXPIRE THREE MONTHS FROM THE DATE MAILED indicated on this form. Failure to timely comply will result in the ABANDONMENT of this application. Extensions of time may be obtained under the provisions of 37 CFR 1.136(a).

1 ☐ Note the attached EXAMINER'S AMENDMENT or NOTICE OF INFORMAL APPLICATION, PTO-152, which discloses that the oath or declaration is deficient. A SUBSTITUTE OATH OR DECLARATION IS REQUIRED.

2. ☑ APPLICANT MUST MAKE THE DRAWING CHANGES INDICATED BELOW IN THE MANNER SET FORTH ON THE REVERSE SIDE OF THIS PAPER.

a. ☐ Drawing informalities are indicated on the NOTICE RE PATENT DRAWINGS, PTO-948, attached hereto or to Paper No. _____. CORRECTION IS REQUIRED.

b. ☐ The proposed drawing correction filed on _____ has been approved by the examiner. CORRECTION IS REQUIRED.

c ☐ Approved drawing corrections are described by the examiner in the attached EXAMINER'S AMENDMENT. CORRECTION IS REQUIRED.

d. ☑ Formal drawings are now REQUIRED

Any response to this letter should include in the upper right hand corner, the following information from the NOTICE OF ALLOWANCE AND ISSUE FEE DUE: ISSUE BATCH NUMBER, DATE OF THE NOTICE OF ALLOWANCE, AND SERIAL NUMBER.

Attachments

☑ Examiner's Amendment
☑ Examiner Interview Summary Record, PTOL-413
☑ Reasons for Allowance
☐ Notice of References Cited, PTO-892
☐ Information Disclosure Citation, PTO-1449
☐ Notice of Informal Application, PTO-152
☐ Notice re Patent Drawings, PTO-948
☐ Listing of Bonded Draftsmen
☐ Other

Stephen Walsh
STEPHEN WALSH
SUPERVISORY PATENT EXAMINER
GROUP 1800

PTOL-37 (REV. 4-89) *

USCOMM-DC 89-3789

part III DETAILED ACTION

Response to Amendment

1. The amendment of 12/1/97 has been entered.

2. The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office Action.

3. The rejection claims 54-76 under 35 U.S.C. §112, first paragraph, are withdrawn in view of Applicants arguments and amendments.

4. The rejection claims 54-76 under 35 U.S.C. § 112, second paragraph, are withdrawn in view of Applicants arguments and amendments.

5. An Examiner's Amendment to the record appears below. Should the changes and/or additions be unacceptable to applicant, an amendment may be filed as provided by 37 C.F.R. § 1.312. To ensure consideration of such an amendment, it **MUST** be submitted no later than the payment of the Issue Fee.

Authorization for this Examiner's Amendment was given in a telephone interview with attorney Matthew Vincent on 30 December 1997.

In the claims:

Cancel claims 1-24, 41, and 49-53

6. The following is an Examiner's Statement of Reasons for Allowance:

The prior art of record does not teach or suggest the instantly claimed *elf-1 polypeptide* and portions thereof having EPH-like receptor binding activity and the recited sequences, because the nearest prior art provides no suggestion of said sequences.

Any comments considered necessary by applicant must be submitted no later than the payment of the Issue Fee and, to avoid processing delays, should preferably accompany the Issue Fee. Such submissions should be clearly labeled "Comments on Statement of Reasons for Allowance."

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Kenneth A. Sorensen at telephone number (703) 305-5377. The examiner can normally be reached on Monday through Friday from 9:00 a.m. to 5:30 p.m.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Stephen Walsh can be reached on (703) 308-2957.

Official papers filed by FAX should be directed to (703) 308-4242. Faxed draft or informal communications with the examiner should be directed to 703)308-0294.

Communications via Internet e-mail regarding this application, other than those under 35 U.S.C. 132 or which otherwise require a signature, may be used by application and should be addressed to [stepehen.walsh@uspto.gov].

All internet e-mail communications will be made of record in the application file. PTO employees do not engage in Internet communications where there exists a possibility that sensitive information could be identified or exchanged unless the record includes a properly signed express waiver of the confidentiality requirements of 35 U.S.C. 122. This is more clearly set forth in the Interim Internet Usage Policy published in the Official Gazette of the Patent and Trademark on February 25, 1997 at 1195 OG89.

Any inquiry of general nature or relating to the status of this application should be directed to the Group receptionist whose telephone number is (703) 308-0196.

Kenneth A. Sorensen, Ph.D.
Examiner
Group 1800

Stephen Walsh
STEPHEN WALSH
SUPERVISORY PATENT EXAMINER
GROUP 1800

Response under Rule 116
Expedited Delivery Requested

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: John G. Flanagan and Hwai-Jong Cheng Serial No.: 08/393,462 Filed: February 27, 1995 For: *EPH Receptor Ligands and Uses Related Thereto*	Attorney Docket No.: HMV-011.02 Group Art Unit: 1812 Examiner: Kenneth A. Sorenson

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D.J.
12/9/
(NE

Assistant Commissioner for Patents
Washington, D.C. 20231

Certificate of First Class Mail

I hereby certify that this correspondence is being deposited with the United States Postal Services as in First Class Mail envelope addressed to: Assistant Commissioner for Patents, Washington, D.C. 20231 on the date set forth below.

November 28, 1997 — By: Matthew P. Vincent
Date of Signature and Mail Deposit

Amendment and Response

Sir:

In response to the Office Action dated May 27, 1997, Applicants submit the following amendments and remarks. An appropriate three-month extension of time, a copy of which is attached hereto, and fee for responding to this action has been filed on November 26, 1997.

Please amend the above-identified application as follows:

In the Claims:

As a courtesy to the Examiner, all of the claims, whether amended or not, are reiterated below.

1 ~~54~~. A substantially pure preparation of an *Elf-1* polypeptide, which polypeptide comprises an *Elf-1* amino acid sequence at least 70 percent identical to the amino acid sequence selected from the group consisting of SEQ ID Nos. 2 and 4, and portions thereof, and which *Elf-1* polypeptide specifically binds to an EPH-type receptor.

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2 ~~55~~. The *Eff-1* polypeptide of claim ~~54~~ 1, wherein said EPH-type receptor is a mek4/sek-type receptor.

3 ~~56~~. The *Eff-1* polypeptide of claim ~~55~~ 2, wherein said EPH-type receptor is selected from the group consisting of mek4-related receptors and sek-related receptors.

4 ~~57~~. The *Eff-1* polypeptide of claim ~~54~~ 1, which polypeptide modifies cellular activities of a cell which expresses an EPH-type receptor.

5 ~~58~~. The *Eff-1* polypeptide of claim ~~57~~ 4, which polypeptide modifies at least one of proliferation, differentiation, cell-cell contact and survival of said cell.

6 ~~59~~. The *Eff-1* polypeptide of claim ~~57~~ 4, which polypeptide stimulates intracellular signal transduction pathways mediated by said EPH-type receptor.

7 ~~60~~. The *Eff-1* polypeptide of claim ~~57~~ 4, which polypeptide antagonizes intracellular signal transduction pathways mediated by said EPH-type receptor.

B' cont 8 ~~61~~. (**amended**) The *Eff-1* polypeptide of claim ~~54~~ 4, wherein said *Eff-1* amino acid sequence includes a Cys_4 motif at least 70 percent identical to a Cys_4 motif **[**is presented in one or both**]** having the sequence of SEQ ID Nos. 2 **[**and**]** or 4.

9 ~~62~~. (**amended**) The *Eff-1* polypeptide of claim ~~54~~ 4, wherein said *Eff-1* amino acid sequence includes a core sequence motif at least 70 percent identical to a core sequence motif **[**represented in one or both**]** having the sequence of SEQ ID Nos. 2 **[**and**]** or 4.

10 ~~63~~. (**amended**) The *Eff-1* polypeptide of claim ~~54~~ 4, which polypeptide comprises a mature polypeptide at least 70 percent identical with a mature polypeptide **[**represented in one or both**]** having the sequence of SEQ ID Nos. 2 **[**and**]** or 4.

11 ~~64~~. The *Eff-1* polypeptide of claim ~~54~~ 4, which polypeptide is post-translationally modified to include a covalently bonded moiety selected from the group consisting of a glycosyl-phosphatidylinositol and a carbohydrate.

12 ~~65~~. (**amended**) A substantially pure preparation of an *Eff-1* polypeptide, which polypeptide comprises a Cys_4 motif at least 70 percent identical to a Cys_4 motif **[**represented in one or

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both] having the sequence of SEQ ID Nos. 2 [and] or 4, which polypeptide specifically binds a mek4/sek-type receptor.

13 ~~66~~. The *Eff-1* polypeptide of claim 12 ~~65~~, which Cys_4 motif is represented in the general formula designated by SEQ ID No. 5.

14 ~~67~~. (**amended**) A recombinant polypeptide comprising an *Eff-1* polypeptide sequence at least 70 percent identical to an amino acid sequence selected from the group consisting of SEQ ID Nos. 2 and 4, and portions thereof, and which *Eff-1* polypeptide [specfically] specifically binds to an EPH-type receptor.

15 ~~68~~. The recombinant polypeptide of claim 14 ~~67~~, which EPH-type receptor is selected from the group consisting of mek4-related receptors and sek-related receptors.

16 ~~69~~. (**amended**) The recombinant polypeptide of claim 14 ~~67~~, which *Eff-1* polypeptide sequence comprises a Cys_4 motif at least 70 percent identical [indentical or homologous] to a Cys_4 motif [represented in one or both] having the sequence of SEQ ID Nos. 2 [and] or 4.

B' cont. 17 ~~70~~. (**amended**) The recombinant polypeptide of claim 14 ~~67~~, which *Eff-1* polypeptide sequence comprises a core sequence motif at least 70 percent identical [or identical] to a core sequence motif [represented in one or both] having the sequence of SEQ ID Nos. 2 [and] or 4.

18 ~~71~~. (**amended**) The recombinant polypeptide of claim 14 ~~67~~, which polypeptide comprises a mature *Eff-1* polypeptide sequence at least 70 percent identical with a mature polypeptide [represented in one or both] having the sequence of SEQ ID Nos. 2 [and] or 4.

19 ~~72~~. The recombinant polypeptide of claim 14 ~~67~~, which polypeptide is post-translationally modified to include a phosphatidylinositol moiety, a carbohydrate, or both.

20 ~~73~~. (**amended**) The recombinant polypeptide of claim 14 ~~67~~, which protein is a fusion protein [further comprising, in addition to said *Eff-1* polypeptide sequence, a second polypeptide sequence having an amino acid sequence unrelated to said *Eff-1* polypeptide sequence].

21 ~~74~~. (**amended**) The recombinant polypeptide of claim 20 ~~73~~, wherein said fusion protein further includes, [as a second polypeptide sequence, a] in addition to said *Eff-1* polypeptide

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sequence, a second polypeptide sequence which functions as a detectable label for detecting the presence of said fusion protein or as a matrix-binding domain for immobilizing said fusion protein.

22-75. (**amended**) The recombinant polypeptide of claim [73] 74 wherein said second polypeptide sequence has an enzymatic activity.

B'. cont. 23-76. An isolated or recombinant polypeptide comprising an *Eff-1* polypeptide sequence at least 90 percent identical with an amino acid sequence represented in the general formula designated by SEQ ID No. 5 and which polypeptide specifically binds to an EPH-type receptor.

Remarks

The pending claims are patentably distinct from the art

Applicants note with appreciation that the pending claims have been deemed to be patentably distinct from the art.

The pending claims comply with 35 U.S.C. §112, first paragraph

A. The Examiner has rejected certain of the pending claims under 35 U.S.C. §112, first paragraph, as failing to provide an enabling disclosure. The Examiner argues

> The instant specification does not enable an artisan to make *Eff-1* proteins or polypeptides that are "at least 90%..." or "at least 70% identical..." to the disclosed *Eff-1* sequences of SEQ ID Nos 1-5 because it does not identify those amino acid residues/base pairs in the amino acid/nucleic acid sequence of *Eff-1* which are essential for its biological activity and structural integrity and those residues which are either expendable or substitutable. Further, no working examples or guidance to make specific sequences are cited that would enable a skilled artisan to produce other non-disclosed *Eff-1* proteins having a biological activity of *Eff 1*, nor are there sufficient prior art teachings to enable one skilled in the art to produce such a peptide, which precludes one from reasonably predicting the result of different modifications to the peptide sequence without an inordinate degree of experimentation.

The Examiner's argument is respectfully traversed. Applicants summarize the arguments as follows:

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- The disclosure of multiple *elf-1* clones from different vertebrates, e.g., ranging from birds to mammals, provides sufficient guidance to claim all vertebrate *elf-1* proteins.
- Combinatorial mutagenesis, exemplary uses of which are described in present application, is an art-recognized approach for generating and rapidly screening large libraries of polypeptide sequences in order to identify homologs of similar activity – and can be undertaken without any a priori knowledge of the effect of any particular amino acid residue on the function of the protein of interest. The application, and the art, provide sufficient guidance for one or ordinary skill in the art to identify non-naturally occurring *elf-1* homologs which are the equivalent of the wild-type proteins in terms of retaining at least certain biological activities. Moreover, such techniques were developed to the point that, at the time the present invention was made, that one skilled in the art would reasonably expect that combinatorial mutagenesis could be performed without any undue experimentation in order to identify *elf-1* homologs.
- The *elf-1* proteins demonstrate cross-species activity, e.g., chicken *elf-1* protein can bind to mouse receptor. These results reasonably suggest to the skilled artisan that the *elf-1* proteins can tolerate abundant mutations without substantially abrogating the biological activity of the protein relative to wild-type *elf-1*, and that these mutational homologs represent equivalents to the native proteins.
- The art details the ability of combinatorial mutagenesis techniques to successfully engineer growth factors and other secreted proteins. There is a general technical similarity between engineering such extracellular and transmembrane proteins as reported in the literature, and the *elf-1* polypeptides of the subject claims.

In toto, one skilled in the art would reasonable conclude that mutagenesis techniques could be readily and successfully applied to identify a wide range of *elf-1* variants retaining at least a portion of the biological activity of a wild-type *elf-1* protein.

Detail of Applicants' rebutting arguments

First, Applicants note that the claims recite the limitation that the polypeptide be capable of specifically binding to an EPH-type receptor. Thus, the claims read only on those variants of the *Elf-1* protein which retain the recited biological activity. Furthermore, Applicants teach two *Elf-1* proteins: a mouse *Elf-1* protein and a chicken *Elf-1* protein. A comparison of the amino acid sequence of these two proteins resulted in the establishment of a general formula, i.e., SEQ ID No. 5, representing *Elf-1* homologs. In addition, the specification describes a variety of different methods for varying the primary sequence of an *Elf-1* protein and selecting those variants for

receptor-binding activity (discussed in further detail below). Similar claim language requiring operability has been held to overcome a potential problem of a claim reading on a broad range of embodiments where, absent the recitation of a particular activity, many of which were allegedly inoperative. See, for example, *Ex parte Mark*, 12 USPQ 1905 (Bd Pat App & Int'f 1989) ("when it is considered that the claims remaining on appeal all require that the mutein produced retain the biological activity of the native protein, we consider the disclosure of this application to be enabling").

Moreover, Applicants note that a long pedigree of cases have held that there is no requirement of *a priori* knowledge of all specific embodiments of the claimed invention. Accordingly, the Examiner's position that predictability of which changes (i.e. deletions, additions or modifications) can be tolerated in the subject *Eff-1* proteins is critical to enablement is contradictory to scope and spirit of such decisions. Enablement is not precluded even if some experimentation is necessary. Applicants contend that the specification provides sufficient guidance with respect to isolating homologs of the subject *Eff-1* proteins having the recited biological activity, e.g., such as allelic variants, homologs from other animals, or homologs generated by mutation, such that a person of ordinary skill in the art could make and use the claimed *Eff-1* proteins without undue experimentation, relying on the specification and knowledge in the art.

Contrary to the Examiner's statement that the "few embodiments" provided in the specification do not enable scope of the claimed proteins, Applicants' teachings clearly enable the skilled artisan to make and use natural and man-made homologs of the *eff-1* polypeptides at least the following reasons. For example, the specification sets forth various protocols which could be used to identify the homologs of the subject *Eff-1* genes in other animals. Additionally, the specification provides further guidance as to the portions of the gene or protein which could be used to derive nucleic acid probes or antibodies, respectively, for isolating such homologs. For instance, the subject application discloses that homologs of the subject *Eff-1* genes can be isolated on the basis of their ability to hybridize under stringent conditions with the human *Eff-1* gene described in the examples. The specification teaches at page 28, paragraph two that alignment of Applicants' *Eff-1* protein with the only two other known EPH receptor ligands, namely B61 and LERK-2, suggests a conserved motif(s). Similarities between these three proteins indicate that, for example, a Cys_4 motif (e.g., Cys-69 through Cys-159 of *Eff-1*) is apparently conserved amongst EPH receptor ligands, and accordingly would be expected to be conserved amongst the family of *Eff-1* ligands. It is respectfully asserted that one skilled in the art would reasonably expect to be able to clone homologs of the subject *Eff-1* genes, such as from other mammalian organisms, using for example the hybridization techniques set forth in the present application, or using other such hybridization techniques as were state-of-the-art at the time the subject application was filed. The skilled artisan

would recognize, for instance, that such conserved motifs as discussed above are useful in designing probes/primers for cloning *Elf-1* homologs from other animals.

Moreover, the target of the subject *Elf-1* proteins, e.g., the EPH-type receptors such as the MEK4 and SEK receptors, are highly conserved in other animals. See, for example, Tuzi et al. (1994) *Br J Cancer* 69:417-421 (attached as Exhibit A). For example, references, such as Tuzi et al., published prior to the priority date of the instant application describe the identification and cloning of members of the EPH receptor superfamily from across a wide spectrum of animals. To illustrate, the Tuzi et al. article suggests that the EPH-type murine receptors MEK4 and SEK, to which *Elf-1* binds, are conserved in other mammals (e.g. humans) as well as avian species (e.g., chicken). The conservation of these receptor proteins amongst such diverse animals would reasonably suggest to the skilled artisan that, in similar fashion, the *Elf-1* protein would also be expected to be conserved across such a wide range of animals, and particularly conserved across the class *Mammalia.*

In addition to the description of methods for cloning homologs in the specification, Applicants describe that a homolog has in fact been cloned using one of these methods. The homolog cloned by Applicants is the chicken *Elf-1* gene and it was cloned by hybridization with a mouse cDNA probe as described at pages 81-82 of the specification under the heading *"G. Cloning of chicken Elf-1 protein, and chicken EPH receptors"*. Moreover, the present application teaches the detection of a human *Elf-1* gene, indicating that the human *Elf-1* gene can also be cloned by hybridization.

Moreover, the Examiner's arguments are also controverted by the fact that Applicants have cloned *Elf-1* homologs from such disparate organisms as mammals and birds. The present application teaches the cloning of a chicken *Elf-1* which is 70% (69.5) identical to the mouse *Elf-1* protein sequence of SEQ ID No. 2. The fact that *Elf-1* could be cloned from organisms as widely separated in evolutionary time would suggest to the skilled artisan that the *Elf-1* gene is conserved among vertebrates to an extent that one skilled in the art would have a reasonable expectation of success in isolating *Elf-1* genes from any vertebrate organism.

Thus, using the *elf-1* sequences provided by the Applicants' from mouse and fish, the skilled artisan can, by no more than routine experimentation, isolate other naturally occurring homologs of the *elf-1* polypeptides. Experimental conditions for genomic library screening and PCR are extensively detailed in the specification and Examples. Furthermore, Applicants describe the use of anti-*elf-1* antibodies to screen cDNA libraries constructed using expression cloning system. Thus, applicants have provided extensive guidelines to be used to identify and sequence naturally-occurring homologs of *elf-1* in multiple species.

Furthermore, the art is replete with examples of applications of PCR techniques to isolate and sequence DNA from distantly related species, including rare and even extinct organisms. In fact, Paabo and co-workers have successfully amplified sequences derived form a 7,000-year-old mummified brain and mitochondrial DNA using these techniques (See Paabo, S. et al. (1988) *Nucl. Acid Res.* 16(20):9775-87; Paabo, S. (1989) *Proc. Natl. Acad. Sci USA* 86: 1939-43). Moreover, the advent of PCR techniques has substantially simplified the cloning of new genes compared to conventional techniques. In fact, PCR techniques allow the cloning of unique sequences in a matter of hours (Saiki, R.K. *et al.* (1988) *Science* 239: 487-91). Moreover, the procedure is easily automated so that hundreds of samples can be amplified each day. Sequencing of the reaction products can be performed directly from the amplified product as described by Wrischnik, L.A. *et al.* (1987) *Nucl. Acid Res.* 15:529-42; Gyllenstein, U.B. and Erlich, H.A. (1988) *Proc. Natl. Acad. Sci* USA 85: 7652-56, which facilitates even further these procedures.

In addition to providing examples describing the isolation of naturally-occurring *elf-1* polypeptides, Applicants have provided extensive guidelines describing methods of generating and screening sets of combinatorial mutants of *elf-1* proteins (and truncation mutants thereof), which are especially useful for identifying potential variant *elf-1* sequences (e.g. homologs), which can act as either agonists or antagonists of *elf-1* activity. For example, the specification provides a degenerate amino acid sequence, SEQ ID No. 5, which represents the amino acids that are conserved between the mouse and the chicken *Elf-1* proteins and which are thus likely to be important in the biological activity of *Elf-1*. According to this sequence a person of skill in the art can produce numerous *Elf-1* homologs having the required biological activity. The specification also provides exemplary derivatives of SEQ ID No. 2 including proteins which lack N-glycosylation sites (e.g. to produce unglycosylated protein), lack N-terminus and/or C-terminus sequence of the *Elf-1* protein or an *Elf-1* polypeptide comprising Cys 69 through Cys 159.

In addition, at page 25, lines 3-11, Applicants explain that the wild-type *Elf-1* protein contains 3 potential glycosylation sites, Asn-38, Asn 170 and Asn-184, which can be mutated. Moreover, the specification discloses preferred soluble forms of the *Elf-1* polypeptide which lack at least the last 15 amino acid residues (truncated at Leu-195). Polypeptides truncated anywhere between Thr-182 to Leu-195 are also contemplated.

The specification also sets forth conservative mutations which a skilled artisan would not consider to have a major effect on the biological activity of the resulting molecule. For example, it is reasonable to expect that an isolated replacement of leucine with isoleucine or valine, an aspartate with glutamate, a threonine with a serine, or replacement of other amino acids with structurally related amino acids would not significantly alter the biological activity of *Elf-1* polypeptide.

Thus, contrary to the Examiner's statements, Applicants have provided sufficient examples of *Elf-1* proteins, as well as teachings regarding which amino acids can be substituted in the *Elf-1* proteins such that the claimed polypeptides retain the required biological activity, i.e., the capability to specifically bind to an EPH-type receptor.

In addition to the arguments set out above, the Examiner's comments are also traversed as being contrary to the teachings in the art. The art (and the instant specification) is replete with examples of combinatorial techniques for identifying variants and fragments of naturally-occurring proteins which retain a particular biological activity of the naturally-occurring proteins. At the time of the instant invention, combinatorial techniques for generating and processing libraries of variants of a protein were routine in the art, even for libraries exceeding a billion different variants. For instance, those skilled in the art would recognize that *Elf-1* variants, including minimal binding domains of the subject *Elf-1* proteins, could be readily isolated by subjecting the protein to, for example, alanine scanning mutagenesis and the like (Lowman et al. (1991) *Biochemistry* 30:10832-10838; and Cunningham et al. (1989) *Science* 244:1081-1085), by linker scanning mutagenesis (Brown et al. (1992) *Mol. Cell Biol.* 12:2644-2652; McKnight et al. (1982) *Science* 232:316); by saturation mutagenesis (Meyers et al. (1986) *Science* 232:613); by PCR mutagenesis (Leung et al. (1989) *Method Cell Mol Biol* 1:11-19); or by random mutagenesis (Miller et al. (1992) *A Short Course in Bacterial Genetics*, CSHL Press, Cold Spring Harbor, NY). The present application specifically teaches the use of such techniques, in the context of high throughput binding assays, in a manner which permits the rapid generation of large libraries of *Elf-1* variants and the isolation from those libraries of *Elf-1* variants which retain the ability to specifically bind a EPH-type receptor.

The specification provides extensive guidelines for generating and screening sets of combinatorial mutants for *Elf-1*. For example, Applicants suggest the use of "panning assays", display techniques on cell surface or viral particles, recombinant phage antibody system, or detectably labeled EPH receptors, such as the *mek4*-AP or *sek*-AP fusion proteins described in Experimental Procedure A, to screen the combinatorial library.

To further illustrate the state of the art, the Examiner's attention is directed to the review article of Gallop et al. (1994) *J Med Chem* 37:1233 (previously made of record). Gallop et al. describes the general state of the art of combinatorial protein libraries throughout the early 1990's, e.g., at the time of the filing date of the instant application. In particular, Gallop et al state at page 1239 "[s]creening the analog libraries aids in determining the minimum size of the active sequence and in identifying those residues critical for binding and intolerant of substitution".

In addition, the Ladner et al. PCT publication WO90/02809, the Goeddel et al. U.S. Patent 5,223,408, and the Markland et al. PCT publication WO92/15679 (previously made of record)

illustrate specific techniques which one skilled in the art could utilize to generate libraries of *Elf-1* variants which can be rapidly screened to identify variants/fragments which retained a particular activity of the murine *Elf-1* protein of SEQ ID No. 2 or SEQ ID No. 4, e.g., such as receptor binding. These techniques are exemplary of the art and demonstrate that large libraries of related variants/truncants can be generated and assayed to isolate particular variants without undue experimentation. The Examiner's attention is also directed to Gustin et al. (1993) *Virology* 193:653 (previously made of record), and Bass et al. (1990) *Proteins: Structure, Function and Genetics* 8:309-314 (previously made of record) which each describe other exemplary techniques from the art which would be recognized as means for generating mutagenic variants of *Elf-1* proteins.

It is plain from the combinatorial mutagenesis art that it was in fact routine for those skilled in the art to engage in large scale mutagenesis of proteins, without any preconceived ideas of which residues were critical to the biological function, and generate wide arrays of variants having equivalent biological activity. *Indeed, it is the ability of combinatorial techniques to screen billions of different variants by high throughout analysis that removes any requirement of a priori understanding or knowledge of critical residues.*

In addition to the above-arguments, Applicants note that the present application teaches examples of cross-species activity, e.g., the *elf-1* proteins could withstand a substantial degree of mutation while retaining biological activities of the wild-type protein. In particular, the Examples describe the ability of the chicken *elf-1* protein to bind to the mouse mek4 receptor. Accordingly, in view of the disparity in sequence between the chicken and mouse *elf-1* proteins, one of ordinary skill in the art would conclude that there was an appreciable amount of "play" with respect to retaining the biological activity of receptor binding of any given *elf-1* protein. That is, it would be reasonably expected that the *elf-1* proteins can each tolerate a substantial number of mutations without substantially abrogating the receptor binding activity of the protein relative to wild-type *elf-1*, and that these mutational homologs represent equivalents to the native proteins.

Moreover, at the time the present application was filed, the art included a variety of publications detailing the ability of combinatorial mutagenesis techniques to successfully engineer growth factors and other secreted proteins, e.g., analogous proteins to *Elf-1*. In light of the general technical similarities between the engineering of such extracellular and transmembrane proteins as reported in the literature, and the *elf-1* polypeptides of the subject claims, those skilled in the art would reasonable conclude that combinatorial mutagenesis techniques could be applied to identify a wide range of *elf-1* variants retaining at least a portion of the bioactivity of a wild-type *elf-1* protein.

The only inventive step(s) required to utilize the *Elf-1* proteins as claimed have already been carried out by the Applicants. Routine screening techniques taught in the specification combined

with those techniques available in the art at the time the present invention was made provide sufficient guidance for generating variants of the native *Elf-1* protein, as well as reducing the *Elf-1* proteins to minimum receptor-binding motifs. Furthermore, the instant specification plainly sets forth a variety of assay formats which one skilled in the art would chose as high throughput screens for detecting, e.g., binding activity. Accordingly, Applicants assert that the specification, in light of the art at the time the present invention was made, is enabling for a sufficient number of other permutations of the subject *Elf-1* proteins to entitle Applicants to the invention as presently claimed.

As the legal basis supporting the §112 rejection set forth in the Office Action, the Examiner also cites *Amgen Inc. v. Chugai Pharmaceutical Co.*, 927 F.2d 1200 (Fed. Cir. 1990), specifically reciting, in part, that

> [c]onception does not occur unless one has a mental picture of the structure of the chemical, or is able to define it by its method of preparation, its physical or chemical properties, or whatever characteristics sufficiently distinguish it...We hold that when an inventor is unable to envision the detailed chemical structure of the same gene so as to distinguish it from other materials, as well as a method of obtaining it, conception has not been achieved until reduction to practice has occurred.

Applicants respectfully traverse the Examiner's objection based on the cited *Amgen* opinion. The cite provided by the Examiner pertains to the establishment of conception of a chemical compound for purposes of priority of an invention under 35 U.S.C. §102(g), as opposed to the issue of lack of enablement under 35 U.S.C. §112 pertinent to this rejection. Enablement under §112 requires that a disclosure must be sufficient to enable the skilled artisan to make and use the claimed invention, as opposed to conception under §102(g) which requires an inventor to have an idea of the invention's structure, as well as possession of an operative method in making it. *See Amgen*, 927 F.2d at 1206, 1212-14. The requirements to comply with these two provisions are completely distinct and should not be used as a basis for this objection.

Furthermore, even the sections of the *Amgen* opinion which address the issue of enablement under §112 are inapplicable to Applicants' case. *See Amgen*, 927 F.2d at 1212-14. Although the *Amgen* court found that the generic DNA sequence claims in plaintiff's patent did not satisfy the enablement requirement under §112, the facts of that case are clearly distinguishable from the Applicants' situation.

Unlike the situation in *Amgen*, Applicants have been able to define a conserved region between *Elf-1* polypeptide and the two other known EPH receptor ligands. As explained above, the conservation of the four cysteine regions suggests that it is essential to the bioactivity of *Elf-1*

polypeptide. (*See* Application at page 28). Thus, unlike *Amgen* where the plaintiffs had only describe a single EPO gene, Applicants have not only provided a description of the *Elf-1* polypeptide set forth in SEQ ID No.2 and in SEQ ID No. 4, but have also provided sufficient teaching as to residues that are expendable and substitutable and those which are critical to obtaining functional homologs of the *Elf-1* polypeptide.

Moreover, in *Amgen*, the claims covered ***all possible analogs*** of the EPO gene and were not limited by the nucleic acid sequence included in the specification. Applicants' claims, however, cover an *Elf-1* nucleic acid encoding a polypeptide that is "at least 70% identical" the amino acid sequence described in one or both of SEQ ID Nos.2 and 4 or hybridizes under stringent conditions to the nucleic acid sequence represented by SEQ ID Nos. 1 or 3. Moreover, each claim requires that the protein be capable of specifically binding to an EPH-type receptor. Thus, Applicants' invention clearly ***does not cover any and all of the possible analogs*** of the *Elf-1* polypeptide.

A second ground for the holding of the *Amgen* court restricting the scope of the generic claim in plaintiff's patent was that little information was disclosed in that specification regarding how to make EPO analogs. In doing so, the court evaluated the state-of-the-art techniques in molecular biology as of 1981, when the facts of the Amgen case commenced. Moreover, the CAFC plainly stated

> In affirming the district court's invalidation of claims 7, 8, 23-27 and 29 under Section 112, ***we do not intend to imply that generic sequences cannot be valid where they are of a scope appropriate to the invention disclosed***'

plainly implying that advances in the art are critical to and *Amgen*-like analysis of enablement. *As described above, the level of expertise of the skilled artisan in 1981 differs substantially to the level of expertise in 1994, the priority date for Applicant's invention.* Unlike the *Amgen* case where techniques in gene cloning and screening of genomic libraries were at their infancy, with the introduction of the polymerase chain reaction (PCR) in the late 1980s, the ease by which the skilled artisan could generate and screen homologs of a given polypeptide improved drastically.

Applicants respectfully point out that the *Amgen* opinion emphasizes that it is not necessary that a patent application test ***all the embodiments*** of an invention; what is necessary is that s/he provide a disclosure sufficient to enable one skilled in the art to carry out the invention commensurate with the scope of his claim. *See Amgen* 927 F.2d at 1213. According to that court, for DNA sequences, that means "***disclosing how to make and use enough sequences to justify a grant of the claims sought***". *Id.* (emphasis added). Thus, the *Amgen* court recognized that §112, first paragraph, does not require that a disclosure teach ***all possible variants*** of a nucleic acid sequence within the scope of the claims. Furthermore, that some experimentation is necessary

does not preclude enablement. *See e.g., In re Angstadt*, 537 F.2d 498, 503.. In fact, the Court of Appeals for the Federal Circuit has announced a test for enablement requiring that the general description set forth such sufficient detailed guidance that one of ordinary skill in the art would have a reasonable expectation of success in carrying out the claimed invention. *See e.g., In re Wright*, 999 F.2d 1557 (Fed. Cir. 1993).

Accordingly, the Examiner's comment that undue experimentation is required to identify ***all possible*** proteins capable of inducing or regulating the listed cellular activities is controverted by the arguments set out above and in light of the amended claims and cited case law.

In addition, Applicants contend that the invention is "pioneering", and as such, Applicants are entitled to claims of broad scope. As demonstrated by the prior art, a large number of members of the EPH family have been identified as *orphan receptors without known ligands*. Thus, Applicants discovery of one of the first known ligands for EPH-type receptors, i.e., *Elf-1*, is a significant advancement in this area. Narrow claims, e.g., to the particular sequences disclosed, would provide no real protection. The level of skill in the art of molecular biology is high and the skilled artisan could easily avoid such claims by simply practicing the routine screening methods described in the specification.

In sum, given the legal requirements under §112, the support provided by the present specification for making and using the provided sequences to isolate homologs of a *Elf-1* polypeptide, in addition to the above-described contemporary knowledge in the art, the ordinary skilled artisan could make and use the presently provided nucleotide sequences using the described methods without undue experimentation. Applicants' disclosure provides considerable direction and guidance on how to practice the invention.

B. The Examiner has also rejected the claims "reciting *Elf-1* whereby *Elf-1* modulates at least one of proliferation, differentiation or survival of a cell" under §112, first paragraph. The Examiner asserts that these claims "are not enabled because the disclosure does not demonstrate a nexus between these processes and *Elf-1*."

Applicants respectfully traverse. Studies of EPH family receptors have implied important roles for these molecules in early vertebrate development. As discussed at page 11 of the specification, *sek* shows notable expression in areas of mouse embryos that demonstrate obvious segmentation, namely the somites in the mesoderm and the rhombomeres of the hindbrain. Hence, it has been suggested that *sek* plays an important role in forming these segmented structures or in determining segment-specific cell properties. Moreover, EPH receptors have been implicated,

by their pattern of expression, in the development and maintenance of nearly every tissue in the embryonic and adult body.

Applicants have provided sufficient evidence of the relationship between EPH receptors, especially *sek* and *mek4*, and *Elf-1* polypeptide. For example, RNA *in situ* hybridization indicates *Elf-1* expression in early development and in areas of obvious segmentation, such as neural tubes and somites. Furthermore, Applicants RAP-*in situ* experiments with *mek4*-AP and *sek*-AP are strikingly complementary to the areas of expression of *Elf-1* demonstrated by RNA *in situ* hybridization. In Example 5, Applicants demonstrate that in several separate developmental fields of the embryo, *Elf-1* and *mek4* and *sek* receptors are expressed in patterns that are adjacent and complementary. Thus, the results in Example 5 imply roles for the interaction of *Elf-1* and these receptors in patterning of these areas.

Moreover, Applicants have demonstrated that *Elf-1* polypeptide is a cell surface ligand with an affinity for *mek4* and *sek* receptors which are also present on cell surfaces. After treatment with phosphantidylinositol-specific phospholipase C, which is an enzyme that cleaves *Elf-1* polypeptides' phosphantidylinosital linkage to the cell surface, Applicants showed a decreased binding of *mek4* and *sek* receptors to the cell surfaces in which *Elf-1* was removed.

Thus, based on the association between *Elf-1* and EPH receptors, specifically *sek* and *mek4*, which have been suggested to participate in the maintenance of spatial arrangements and in generating or maintaining an array of different vertebrate tissues, a skilled artisan has been provided with sufficient evidence to infer a nexus between the *Elf-1* polypeptide and these functions.

Furthermore, the specification provides techniques for modulating the proliferation, differentiation and/or survival of a cell. . In the paragraph bridging pages 47 and 48 of the specification, Applicants teach that methods of contacting cells with *Elf-1* agonist or antagonist may induce differentiation, enhance survival, and/or promote proliferation of a cell responsive to an *Elf-1* protein. As discussed above, Applicants enable the ordinary skilled artisan to obtain homologs of the *Elf-1* polypeptide. Applicants, further, teach that each of these homologs can subsequently be screened for further biological activities in order to differentiate agonists and antagonists. Methods of screening for homologs, such as detecting autophosphorylation of an EPH receptor in response to a homolog (see, for example, Millauer et al. (1993) *Cell* 72:835 846) are provided in the specification. (*See* Specification page 40).

Thus, Applicants have provided sufficient evidence and guidance so that a person of skill in the art could not only infer the relationship between *Elf-1* polypeptide and the properties recited in the claims, but also provides guidance on how to obtain agonist and antagonists of *Elf-1*

polypeptide which will modulate these functions. Therefore, Applicants respectfully request that the Examiner withdraw these rejections.

For the above-described reasons, Applicant respectfully request that the Examiner reconsider and withdraw the §112, first paragraph rejection.

The pending claims comply with 35 U.S.C. §112, second paragraph

The Examiner rejected the pending claims under 35 U.S.C. §112, second paragraph "as being indefinite for failing to point out and distinctly claim the subject matter the Applicant regards as the invention".

The Examiner's rejection of claim 76 for using the language "at least 90% identical or *homologous...*" is not understood. Claim 76 recites "...at least 90 percent identical with...". Accordingly, the Examiner's arguments concerning the term homologous are not applicable to that claim.

The Examiner has also rejected the pending claims for use of such terms as "EPH-type receptor" or "mek4-related receptor" or "sek-related receptor" or "mek-type" or "sek-type" or mek4/sek-type receptor", because

> no precise definition is recited for what they delimit or constitute in the body of the specification and the applicant doe not clearly teach or refer to any means for obtaining 'EPH-like, EPH-type, Sek-related, mek4-related, mek4/sek-type' sequences.

Applicants respectfully submit that, among the above-cited terms recited by the Examiner, the claims recite only the following terms: "EPH-type receptor", "mek4/sek-type receptor", "mek4-related receptor", and "sek-related receptor", and that these terms are defined in the specification, e.g., in the paragraph bridging pages 13 and 14. Accordingly, "EPH-type receptor" refers to a discrete group of receptors related by homology and easily recognizable, e.g., they are typically characterized b an extracellular domain containing a characteristic spacing of cysteine residues near the N-terminus and two fibronectin type III repeats. The specification further teaches that "mek4/sek type receptors" refers to a closely related subgroup of the EPH receptor family, which subgroup includes: the "mek4-related receptors" such as *mek4*, *cek4*, *hek* and *tyro4*; the "sek-related receptors" such as *sek*, *cek8*, *pagliaccio*, *tyro1* and *rtk1*; as well as other phylogentically related homologs such as *eek*, *bsk*, *ehk1*, *ehk2*, and *cek7*. Accordingly, since the terms relating to EPH receptors used in the claims are defined in the specification, rejection of these claims as being indefinite should be withdrawn.

The Examiner rejected claims 61 and 65, which recite "Cys_4 motif" or "Cys_4 motif...general formula", because "these terms are vague and indefinite because no precise definition is recited for what they delimit or constitute in the body of the specification and the applicant does not clearly teach or refer to any means for obtaining a 'Cys_4 motif' or 'Cys_4 motif...general formula' bearing sequences". Applicants respectfully submit that these terms are defined in the specification. Accordingly, the specification teaches, e.g., at page 12, fourth paragraph, that "Cys_4 motif" refers to a conserved motif consisting of four cysteine residues which are apparently conserved with approximately the same characteristic spacing within the primary sequence of each of the known EPH receptor ligands. The specification also teaches that in exemplary *Elf-1* polypeptides, the Cys_4 motif is represented by residues 69-159 of SEQ ID No. 2 (*muElf-1*), by residues 61-150 of SEQ ID No. 4 (*chElf-1*), and residues 39-129 of SEQ ID No. 5.

The Examiner rejected claims 54-64 and 67-71, because these claims use language such as "a portion thereof". Applicants respectfully submit that the claims are drawn polypeptides which bind specifically to an EPH-type receptor. Thus, the scope of the subject matter encompassed by claims reciting "a portion thereof" is limited by the fact that the claimed polypeptide must be capable of binding to an EPH-type receptor. Accordingly, the claims reciting "a portion thereof" are not indefinite and Applicants respectfully request that this rejection be reconsidered and withdrawn.

The rejection of claims 66 and 76 for use of the phrase "represented in the general formula designated by" is traversed. While Applicants thank the Examiner for the useful suggestions for claim amendments, it is noted that the suggested phrase of "having the sequence of SEQ ID No. 5" would be incorrect for these two claims. SEQ ID No. 5 is a degenerate, general formula, not a discrete, single sequence.

The above-amendments are believed to obviate the Examiner's remaining rejections of the pending claims as detailed in the outstanding office action at page 11, line 9 through page 12, line 14. However, Applicants note that the amendment of claims 61-63, 65 and 69-71 are made with the on the grounds that the Examiner's suggestion of the phrase "having the sequence of SEQ ID No." was made to the Applicant with the express understanding that, when the claim refers to a portion of the full length protein (such as the Cys4 or core motifs), that is the only portion of the recited sequence listing which is limiting. If this understanding is in error, the Examiner should inform the Applicants so that they might withdraw those particular amendments.

Conclusion

In view of the above amendments and remarks, it is believed that this application is in condition for allowance. If a telephone conversation with Applicant's Attorney would expedite prosecution of the above-identified application, the Examiner is urged to call the (617) 832-1000.

Respectfully submitted,
FOLEY, HOAG & ELIOT LLP

By: _____
Matthew P. Vincent
Reg. No. 36,709

Date: 28 November 1997
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Boston, MA 02109
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Appendix A

```
RESULT   7
US-08-308-814-1
; Sequence 1, Application US/08308814
; Patent No. 6268476
;  GENERAL INFORMATION:
;    APPLICANT:  Flanagan, John G.
;    APPLICANT:  Cheng, Hwai-Jong
;    TITLE OF INVENTION:  EPH Receptor Ligands, and Uses Related
;    TITLE OF INVENTION:  Thereto
;    NUMBER OF SEQUENCES:  2
;    CORRESPONDENCE ADDRESS:
;      ADDRESSEE:  LAHIVE & COCKFIELD
;      STREET:  60 State Street
;      CITY:  Boston
;      STATE:  MA
;      COUNTRY:  USA
;      ZIP:  02109
;    COMPUTER READABLE FORM:
;      MEDIUM TYPE:  Floppy disk
;      COMPUTER:  IBM PC compatible
;      OPERATING SYSTEM:  PC-DOS/MS-DOS
;      SOFTWARE:  ASCII(txt)
;    CURRENT APPLICATION DATA:
;      APPLICATION NUMBER:  US/08/308,814
;      FILING DATE:  19-SEP-1994
;      CLASSIFICATION:  435
;    ATTORNEY/AGENT INFORMATION:
;      NAME:  Vincent, Matthew P.
;      REGISTRATION NUMBER:  36,709
;      REFERENCE/DOCKET NUMBER:  HMI-011
;    TELECOMMUNICATION INFORMATION:
;      TELEPHONE:  (617) 227-7400
;      TELEFAX:  (617) 227-5941
;  INFORMATION FOR SEQ ID NO:  1:
;    SEQUENCE CHARACTERISTICS:
;      LENGTH:  1515 base pairs
;      TYPE:  nucleic acid
;      STRANDEDNESS:  both
;      TOPOLOGY:  linear
;    MOLECULE TYPE:  cDNA
;    FEATURE:
;      NAME/KEY:  CDS
;      LOCATION:  [illegible]
;    FEATURE:
;      NAME/KEY:  [illegible]
;      LOCATION:  [illegible]
US-08-308-814-1


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Qy      61 CTGAGCGCTGTGGGTGATGCGGGCGGCTATACCGTGGAGGTGAGCATCAACGACTACCTG 120
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Qy     121 CATATCTACTGCCCACACTACGGGGCGCCGCTGCCCCCGGCTGAGCGCATGGAGCGGTAC 180
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us-08-538-709-1_copy_1_552.rni

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Qy     181 ATCCTGTACATGGTGAATGGTGAGGGCCACGCCTCCTGTGACCACCGGCAGCGAGGTTTC 240
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Qy     241 AAGCGCTGGGAATGCAACCGGCCCGCAGCGCCCGGGGGACCCCTCAAGTTCTCAGAGAAG 300
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Qy     301 TTCCAACCTCTTCACCCCCTTTTCCCTGGGCTTTGAGTTCCGGCCTGGCCACGAATACTAC 360
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Qy     361 TACATCCTGTGCCACACCTCCCAACCTCGTGGACCGACCCTGCCTGCGACTCAAGGTTTAT 420
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Appendix B

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; Sequence 1, Application US/08308814
; Patent No. 6268475
; GENERAL INFORMATION:
;    APPLICANT: Flanagan, John G.
;    APPLICANT: Cheng, Hwai Jong
;    TITLE OF INVENTION: EPH Receptor Ligands, and Uses Related
;    TITLE OF INVENTION: Thereto
;    NUMBER OF SEQUENCES: 2
;    CORRESPONDENCE ADDRESS:
;      ADDRESSEE: LAHIVE & COCKFIELD
;      STREET: 60 State Street
;      CITY: Boston
;      STATE: MA
;      COUNTRY: USA
;      ZIP: 02109
;    COMPUTER READABLE FORM:
;      MEDIUM TYPE: Floppy disk
;      COMPUTER: IBM PC compatible
;      OPERATING SYSTEM: PC-DOS/MS-DOS
;      SOFTWARE: ASCII(text)
;    CURRENT APPLICATION DATA:
;      APPLICATION NUMBER: US/08/308,814
;      FILING DATE: 19-SEP-1994
;      CLASSIFICATION: 435
;    ATTORNEY/AGENT INFORMATION:
;      NAME: Vincent, Matthew P.
;      REGISTRATION NUMBER: ...
;      REFERENCE/DOCKET NUMBER: HM ...
;    TELECOMMUNICATION INFORMATION:
;      TELEPHONE: (617) 227-...
;      TELEFAX: (617) 2..-...
;  INFORMATION FOR SEQ ID NO: 1:
;    SEQUENCE CHARACTERISTICS:
;      LENGTH: 1615 base pairs
;      TYPE: nucleic acid
;      STRANDEDNESS: both
;      TOPOLOGY: linear
;    MOLECULE TYPE: cDNA
;    FEATURE:
;      NAME/KEY: CDS
;      LOCATION: 10..636
;    FEATURE:
;      NAME/KEY: sig_peptide
;      LOCATION: 10..69
US-08-308-814-1

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    34 alSerIleAsnAspTyrLeuAspIleTyrCysProHisTyrGlyAlaPro 50
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    51 LeuProProAlaGluArgMetGluArgTyrIleLeuTyrMetValAsnGl 67
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    67 yGluGlyHisAlaSerCysAspHisArgGlnArgGlyPheLysArgTrpG 84
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   101 PheGlnLeuPheThrProPheSerLeuGlyPheGluPheArgProGlyHi 117
       |||||||||||||||||||||||||||||||||||||||||||||||||||
   385 TTCCAACTCTTCACCCCCTTTTCCCTGGGCTTTGAGTTCCGGCCTGGCCA 434

   117 sGluTyrTyrTyrIleSerAlaThrProProAsnLeuValAspArgProC 134
       |||||||||||||||||||||||||||||||||||||||||||||||||||
   435 CGAATACTACTACATCTCTGCCACACCTCCCAACCTCGTGGACCGACCCT 484

   134 ysLeuArgLeuLysValTyrValArgProThrAsnGluThrLeuTyrGlu 150
       |||||||||||||||||||||||||||||||||||||||||||||||||||
   485 GCCTGCGACTCAAGGTTTATGTGCGTCCAACCAATGAGACCCTGTATGAG 534

   151 AlaProGluProIlePheThrSerAsnSerSerCysSerGlyLeuGlyGl 167
       |||||||||||||||||||||||||||||||||||||||||||||||||||
   535 GCTCCAGAGCCCATCTTCACCAGTAACAGCTCCTGCAGCGGCCTGGGTGG 584

   167 yCysHisLeuPheLeuThrThrValProValLeuTrpSerLeuLeuGlyS 184
       |||||||||||||||||||||||||||||||||||||||||||||||||||
   585 CTGCCACCTCTTCCTCACCACCGTCCCTGTGCTGTGGTCCCTTCTGGGCT 634

   184 er 184
       ||
   635 CC 636
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